Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biological Control 32 (2005) 419–426
Biological Control
www.elsevier.com/locate/ybcon

Effect of dietary selenium supplementation on resistance to baculovirus infection

Holly J.R. Popham[a,*], Kent S. Shelby[a], Thomas W. Popham[b]

[a] USDA, Agricultural Research Service, Biological Control of Insects Research Laboratory, Columbia, MO 65203, USA
[b] USDA, Agricultural Research Service, Southern Plains Area, Stillwater, OK 74075, USA

Received 10 September 2004; accepted 26 December 2004

Abstract

We have examined the effects of dietary selenium (Se) supplementation on larval growth and immunocompetence of the lepidopteran pest, the cabbage looper, *Trichoplusia ni*. Supplementation of the diet of *T. ni* larvae with 10–20 ppm Se resulted in a 1 day delay in pupation. The effects of the addition and/or removal of dietary Se on total Se bioaccumulation and sequestration were determined by neutron activation analysis of pupae. Early penultimate instar larvae moved from selenium containing diet to basal diet lost total pupal Se content down to the level of those fed basal diet. Conversely, larvae moved from basal diet to diet containing additional Se rapidly attained pupal Se levels comparable to larvae fed Se throughout larval development. Therefore, dietary Se is rapidly accumulated or lost during larval development, but significant amounts are sequestered from diet into pupae. Larvae were reared on diet supplemented with 5 or 10 ppm Se until the onset of the penultimate instar then infected per os with increasing concentrations of the fatal baculovirus *Autographa californica* multiple nucleopolyhedrovirus (*Ac*MNPV). Larvae fed Se in the penultimate and ultimate instars were more resistant to viral infection than larvae not fed Se in the final instars. This study indicates that dietary Se levels rapidly impact Se assimilation and sequestration and that tissue Se levels are an important factor in resistance to AcMNPV infection in larval *T. ni*.
Published by Elsevier Inc.

Keywords: *Trichoplusia ni*; *Autographa californica* nucleopolyhedrovirus; Selenium; Biological control; Immunocompetence; Nutritional immunology

1. Introduction

Herbivorous insects encounter a range of dietary nutrients, antioxidants, co-factors, and toxic plant secondary metabolites which affect their development, reproduction, and behavior (Schultz, 2002). In their natural environment, insect populations are subjected to a withering onslaught of microbial pathogens and parasites. Maintaining a vigorous immune defense against pathogens may come at a significant cost to fitness via the diversion of nutritional resources from growth and reproduction (Rolff and Siva-Jothy, 2003). Although many plant derived compounds have demonstrated adverse effects on insect fitness, no plant derived compounds have been identified which impact the immunocompetence of herbivorous insects. Abiotic and biotic stress factors, fasting or specific nutritional deficiencies (i.e., malnutrition) may weaken the immune system enabling opportunistic infection (Rolff and Siva-Jothy, 2003). For example, the micronutrient selenium (Se) has been demonstrated to play a vital role in the immunocompetence of vertebrates (Beck et al., 2004). Se is a cofactor required for the activity of a number of selenoenzymes involved in the stress response, and the maintenance of high tissue antioxidant levels, which may contribute to a more robust antimicrobial and antiviral defense (Beck et al., 2004). This suggested to us that dietary Se may also play a role in insect immune responses. Our artificial diet used to rear *Trichoplusia ni*

* Corresponding author. Fax: +1 573 875 4261.
E-mail address: pophamh@missouri.edu (H.J.R. Popham).

1049-9644/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.biocontrol.2004.12.011

(Hübner) at our laboratory for three decades contained no added Se, resulting in a colony naturally depleted of this element.

Immunocompetence in insects is generally inferred from the survival of an infective challenge, from the circulating levels of antimicrobial peptides, lysozyme, encapsulation by immune cells, or melanization by the enzyme phenoloxidase (Rolff and Siva-Jothy, 2003). The latter two activities have been implicated in resistance to baculovirus infection (Clarke and Clem, 2003; Popham et al., 2004; Trudeau et al., 2001; Washburn et al., 1996). We report that dietary Se levels available to herbivorous insects may play a pivotal role in the resistance of lepidopteran larvae to a normally fatal baculovirus challenge.

2. Materials and methods

2.1. Insects

Trichoplusia ni (Lepidoptera: Noctuidae) larvae obtained from the BCIRL Insectary were reared on an artificial wheat germ based diet under a photoperiod of 14 h:10 h (L:D) at 55% relative humidity at 28 °C. The colony of *T. ni* has been maintained at BCIRL on a meridic diet for over three decades. This diet formulation contains Wesson's salt mixture minerals (Wilkinson et al., 1972) with no added Se and will subsequently be referred to as "basal diet." The low amount of Se present may be contributed predominantly by the wheat germ added to the diet (Anon, 2001). Thus, a viable colony of experimental insects naturally depleted of Se for many generations was fortuitously available for experimentation.

2.2. Selenium content determination

Groups of three pupae or larvae were killed by freezing, placed in pre-tared vials, and oven-dried at 65 °C. Dry weights were calculated. Se determinations were performed by the University of Missouri Research Reactor by instrumental neutron activation analysis using a modification of the method described in McKown and Morris (1978). Pupae were chosen for analysis because larvae completely void midgut contents prior to pupation, eliminating any contribution of diet contents within the digestive system to measured Se levels. Due to the expense of this technique, a single group of three pupae was used for each analysis. Se concentrations are expressed as μg Se/g dry weight (ppm).

2.3. Weight determination studies

Larvae were exposed to Se by the incorporation of differing concentrations of Na_2SeO_3 (Sigma Chemical, St. Louis, MO) directly into the diet during mixing. Three feeding regimes were tested at each of five concentrations of Se: (1) Se-supplemented diet present throughout the entire larval stage until pupation (Se/Se), (2) Se-supplemented diet present until the early fourth instar followed by transfer to basal diet until pupation (Se/NonSe or depletion), or (3) basal diet before the onset of the fourth instar followed by transfer to Se-supplemented diet until pupation (NonSe/Se or repletion). Control larvae were reared on basal diet containing no additional Se. Prior to the onset of the fourth instar, all larvae were fed diet dispensed into individual wax cups in 125 ml aliquots. A sheet of freshly oviposited *T. ni* eggs was stapled to the lids and larvae were allowed to develop in groups of approximately 70–100. At the onset of the fourth instar, larvae were manually transferred to fresh diet in wax cups with the appropriate Se regime and allowed to develop until pupation. Ten larvae were weighed from each of five cups for each Se concentration beginning in the second instar and the weights averaged. Groups of larvae were weighed daily and then discarded (to minimize the effect of handling on growth and survival) until more than 50% of the larvae had pupated. Statistical comparisons were made with the Tukey multiple comparison procedure when a significant ANOVA value was found ($P < 0.05$) (SigmaStat, Systat Software, Point Richmond, CA).

2.4. Viral infections

Virus was produced by per os infection of *T. ni* larvae with polyhedra from the L1 variant of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) (O'Reilly et al., 1992) and the resulting polyhedra were isolated and sucrose gradient purified (McIntosh and Ignoffo, 1983). For the larval bioassays, newly molted fourth instar larvae were infected per os by the droplet feeding method (Hughes et al., 1986) as modified (Slavicek et al., 1999) with concentrations ranging from 1×10^3 to 1×10^6 polyhedra/ml of AcMNPV. Larvae were presented with a 5 μl droplet consisting of virus suspended in water, sucrose, and blue food-coloring. After imbibing, larvae were placed in individual cells with diet. Larvae were monitored twice daily for death for 10 days and the times were recorded. Two bioassays were conducted for each of the three Se regimes.

2.5. Statistical analysis of bioassays

For the six experiments (EXP), two for each combination of Se concentration (SC) and a range of viral concentrations (VC), the cumulative number of dead insects and the elapsed time (T) were computed for each time of observation. An additional variable hour class (HC) was created whereby each observation was classified in 24 h increments, i.e., 0–36 was 24, 36–60 was

48, and 228–252 was 240. Using SAS PROC PROBIT (SAS Institute Inc., 1999), the probit (cumulative dead/initial number of insects) was computed with SC, VC, T, SC*VC, SC*T, VC*T, and SC*VC*T in the model for each experiment of each Se regime (SR). Since every experiment displayed a three-way interaction between the Se concentration, the viral concentration, and time post-infection that was significant, PROC PROBIT was used to compute the viral LC_{50} for all the combinations of EXP, SR, SC, and HC. A regression was fitted to the LC_{50}s to provide a surface representing the effect of the virus on the insects in the presence of differing Se treatments over time. The median time response or LT_{50} (time when 50% of the larvae in a group had died) was obtained by running PROC PROBIT for each combination of SR, SC, and VC with only elapsed time (T) as the independent variable in the model and the probit (cumulative dead/total dead) as the dependent variable.

3. Results

3.1. Effect of Se concentration on growth and development

Whole body larval Se was determined from fifth instar *T. ni* larvae fed basal diet to be 4.2 ± 0.6 ppm dry wt ($n = 5$ groups of three larvae each) which is approximately 1.4 ppm Se/larva. Since only trace amounts of Se are present in *T. ni* larvae or the artificial diet, a preliminary range-finding experiment was conducted to determine the concentration of $NaSeO_3$ which could be added to the lepidopteran diet without deleterious effects on larval development or mortality. Larval weights were recorded from third instar larvae allowed to feed on diet with increasing levels of Se (1–100 ppm) since hatching. We found that dietary supplementation with greater than 25 ppm Se inhibited growth of *T. ni* larvae by 40% based on larval weight. Feeding on diet containing 50 ppm Se resulted in a 62% inhibition of larval growth which was subsequently inhibited by 75% at 100 ppm Se (data not shown). The growth of larvae fed on diets containing from 1 to 10 ppm Se was not significantly different from those fed basal diet (ANOVA, $p = 0.08$) (data not shown). Therefore, in subsequent experiments a range of dietary Se concentrations from 5 to 20 ppm was chosen as a subtoxic range of Se that could be incorporated into the diet without deleterious effects on larvae.

Larval weights were monitored daily in larvae fed on three different regimes of increasing levels of Se (Fig. 1). Higher concentrations of Se in diet (10 and 20 ppm) fed continuously to larvae (Se/Se) caused the growth to lag behind larvae fed 0, 1, or 5 ppm Se (Fig. 1A). However, larvae fed higher concentrations of Se (10 and 20 ppm) until the fourth instar and then moved to basal diet

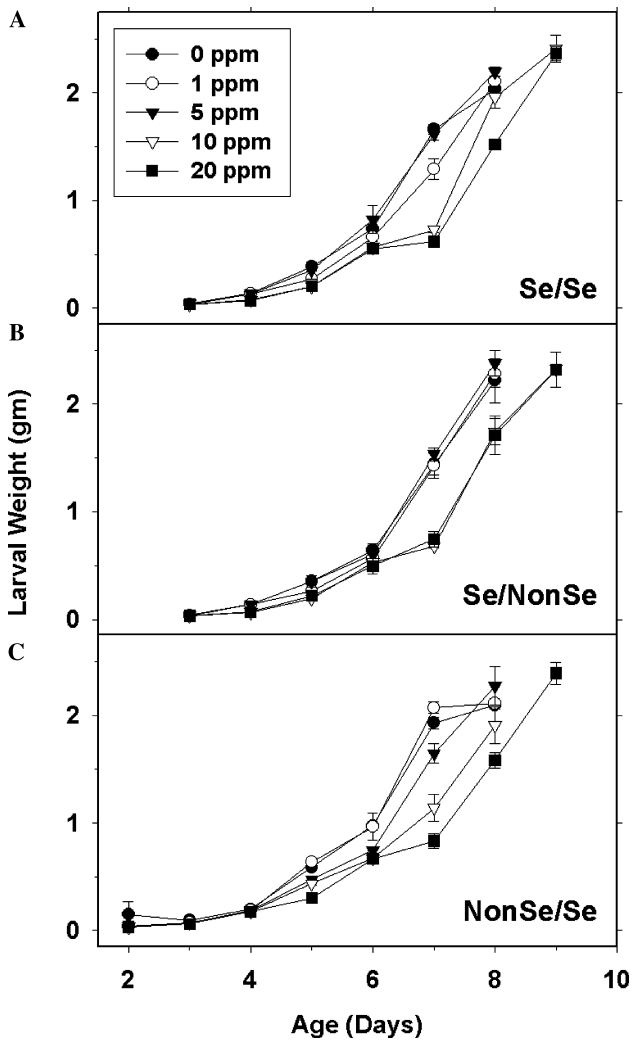

Fig. 1. Larvae were raised in groups on diet with or without selenium then transferred as a group to new diet at the onset of the fourth instar (day 4). A subset of larvae were weighed daily from the second instar until pupation ($n = 5$, mean ± SE). (A) Weights of larvae fed Se-supplemented diet throughout development (Se/Se). (B) Effect of dietary Se depletion on weight. Weights of larvae reared on Se-containing diet then transferred to basal diet at the first day of the fourth instar (Se/NonSe). (C) Effect of dietary Se repletion on weight. Weights of larvae reared on basal diet then transferred to Se-supplemented diet at the first day of the fourth instar (NonSe/Se).

(Se/NonSe) caused the larval growth to lag significantly behind larvae fed 0–5 ppm Se from day 7 of development (Fig. 1B). Larvae fed basal diet until the fourth instar then fed 10 or 20 ppm Se diet (NonSe/Se), lagged behind those fed 0, 1, or 5 ppm Se but began to catch up by the late fifth instar (Fig. 1C). As with the Se/Se fed larvae, NonSe/Se larvae fed 10 ppm caught up in growth by the end of the fifth instar though larvae fed 20 ppm Se lagged a day behind in growth.

Pupae from the above experiment were weighed within 24 h of pupation (Fig. 2). In larvae fed diet containing different amounts of Se throughout development (Se/Se), there was no significant difference in pupal weights

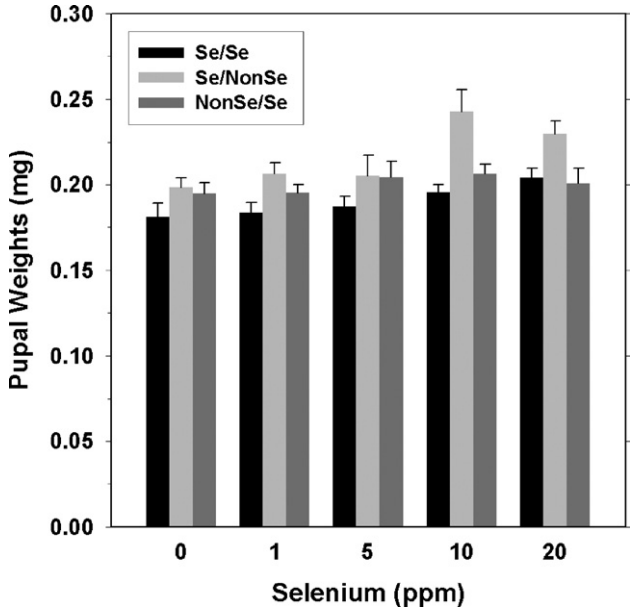

Fig. 2. Final pupal weights attained by *T. ni* larvae were only slightly affected by dietary selenium feeding (Se/Se), depletion (Se/NonSe) or repletion (NonSe/Se). Pupae from the larval weight study shown in Fig. 1 were collected and weighed soon after pupation ($n = 6$, mean ± SE). Larvae were raised in groups on diet with or without Se then transferred as a group to new diet at the onset of the fourth instar (day 4).

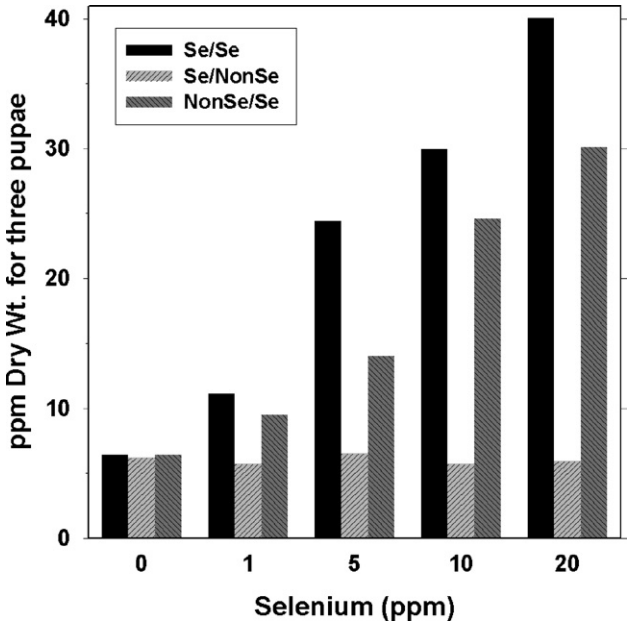

Fig. 3. Pupal selenium sequestration from diet during larval development. Total Se levels were greatly impacted by dietary Se feeding, depletion or repletion. Se levels in pupae were determined by instrumental neutron activation analysis. Each bar represents a group of three pupae.

($P = 0.10$). The same was true for pupae resulting from larvae fed basal diet then moved to diet containing different amounts of Se (NonSe/Se) ($P = 0.10$). However, there was a slight difference in the mean pupal weight attained by larvae fed Se added to their diet and then moved to basal diet (Se/NonSe) ($F = 3.928$, *df* 4.18, $P = 0.018$). The group fed 10 ppm Se diet had a statistically higher mean pupal weight than those that were fed only basal diet ($F = 12.663$, *df* 1.9, $P = 0.006$).

Neutron activation analysis was used to determine the total amount of Se in pupae. Five groups of three pupae fed basal diet during larval development were analyzed to determine the variation between samples with this technique. The amount of Se was 6.6 ± 0.1 ppm Se dry wt (mean ± SE) for three pupae. The amount of Se accumulated by larvae during the course of feeding on Se-supplemented diets was also determined for one group of three pupae from larvae fed the three different regimes of Se (Fig. 3). The amount of Se in pupae continuously fed Se during the larval stage increases with the level of Se fed. However, the amount of Se in Se/NonSe pupae is the same for all levels of exposure and appears to be the same as larvae fed only basal diet. The Se level in NonSe/Se pupae goes up rapidly depending on the amount of Se incorporated into the diet. The amount of Se in pupae fed diet containing Se throughout larval development is more than that in pupae fed diet containing Se only later in larval development.

3.2. Effect of Se concentration on viral infection

Larvae fed on the three regimes of Se were challenged with baculovirus infection at the onset of the fourth instar with varying concentrations of AcMNPV. A regression surface was generated by SAS PROC PROBIT based on the Se level, the time post-infection, and the predicted concentration that killed 50% of larvae (LC_{50}) for each treatment based on larval death over each 24 h period (Fig. 4). For both the Se/Se and the NonSe/Se, the LC_{50} increases as the amount of Se increases at 96 h. This trend is less marked for each 24 h period afterward as fewer larvae die. For Se/NonSe, the LC_{50}s show very little difference as the amount of Se increases. In brief, dietary Se repletion immediately following baculovirus challenge (i.e., transfer of larvae from basal diet to Se-supplemented diet) provided a protective effect against infection equivalent to that of maintenance on Se-supplemented diet throughout the entire experiment (Fig. 4). Dietary Se depletion following infection (i.e., transfer of larvae from Se containing diet to basal diet) resulted in a rapid loss of any protective effect of dietary Se, as Se levels present in the midgut and other tissues declined. Taken together, these data indicate that adequate dietary Se levels, when provided to fourth instar *T. ni* larvae during the early phase of per os baculovirus infection, had a protective effect against AcMNPV.

The effects of dietary Se on trends in the time to death, or lethal time 50% (LT_{50}) were also examined.

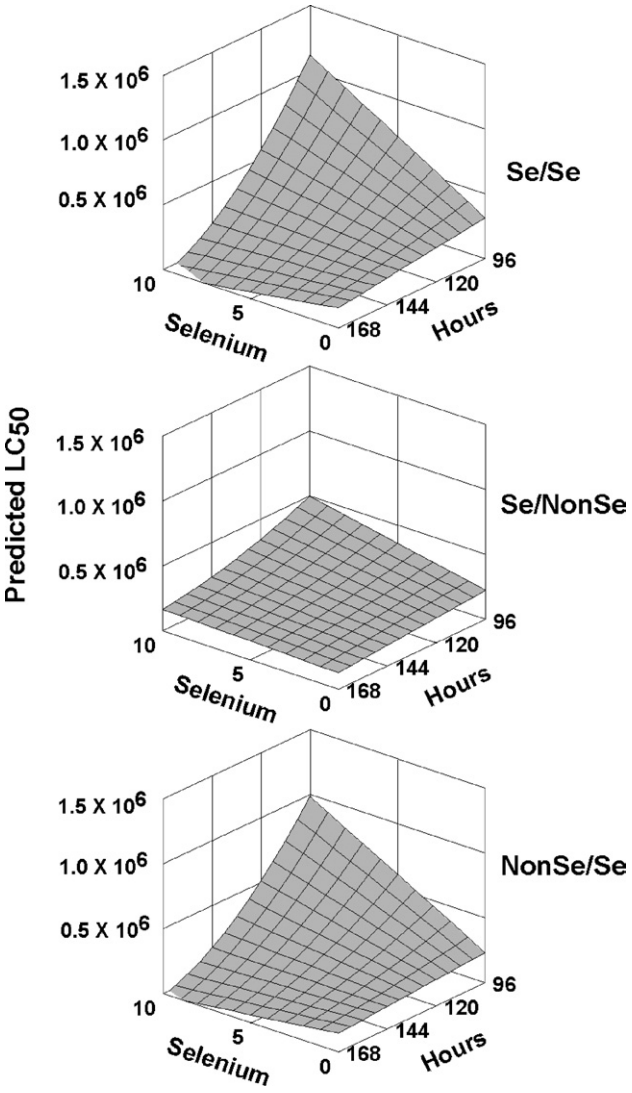

Fig. 4. Dietary supplementation with 0, 5 or 10 ppm selenium in the developmental period before the fourth instar results in varied mortality when larvae are orally infected with baculovirus. A regression was fitted to the LC_{50}s of each Se regime to provide a surface representing the effect of the virus on the insects in the presence of differing Se treatments over time: Se/Se, $LC_{50} = 521864 + 439520 \times Se - 2197.29 \times Hr - 5076.45 \times Se \times Hr + 13.93 \times Se \times Hr^2$, $R^2 = 0.65$; Se/NonSe, $LC_{50} = 355048 + 118522 \times Se - 1369.11 \times Hr - 1301.28 \times Se \times Hr^2$, $R^2 = 0.27$; and NonSe/Se, $LC_{50} = 340207 + 390747 \times Se - 1177.07 \times Hr - 4431.04 \times Se \times Hr + 11.95 \times Se \times Hr^2$, $R^2 = 0.44$.

Because a similar trend was seen for all Se regimes, the LT_{50}s for each Se regime were combined and plotted by viral concentration (Fig. 5). As expected, LT_{50}s declined gradually as the concentration of virus was increased. At the lowest viral concentration, low mortality gave erratic LT_{50}s as expected. No effect of dietary Se concentration on LT_{50}s was observed in the middle range of virus concentrations. However, the LT_{50}s for the larvae fed 5 ppm Se were significantly lower for both of the highest virus concentrations. At a concentration of 7×10^4 polyhedra/ml, the LT_{50}s were 125.8 ± 6.6 h, 108.3 ± 5.0 h, and 124.1 ± 8.4 h (mean $\pm$ SE), respectively, for 0, 5, and 10 ppm Se. At 2.5×10^5 polyhedra/ml, the LT_{50}s were 117.7 ± 6.8 h, 96.2 ± 1.8 h, and 112.2 ± 7.7 h, respectively, for 0, 5, and 10 ppm Se. This suggests that the beneficial effect of 5 ppm dietary Se on mortality may be slightly countered by faster death following infection; but only at the very highest levels of baculovirus exposure. This effect was not also observed at 10 ppm dietary Se.

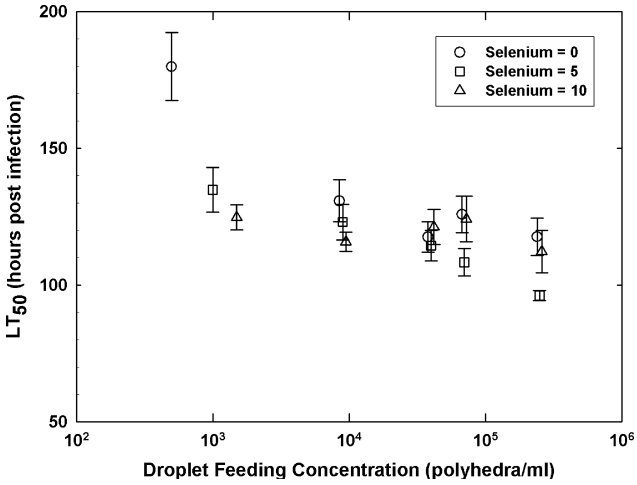

Fig. 5. The median time response or LT_{50} decreased at higher viral concentrations in the 5 ppm Se fed insects. The LT_{50} was calculated by SAS PROC PROBIT for each concentration of each individual experiment and then averaged by dose for all Se regimes (mean $\pm$ SE).

4. Discussion

Adequate dietary levels of the micronutrient Se are necessary for survival, whereas higher levels of Se can be toxic to insects (Jensen and Trumble, 2003). High soil Se content, occurring naturally, as well as the accumulation of Se in soils caused by irrigation, may impact populations of herbivores negatively by interference with molting (Dietrich et al., 1987; Jensen and Trumble, 2003), or by more subtle population effects such as feeding deterrence (Banuelos et al., 2002; Hanson et al., 2003, 2004; Trumble et al., 1998; Vickerman et al., 2002a, 1999, 2004), delayed development (increasing larval exposure to biotic and abiotic mortality factors), or interference with oviposition and reproduction (Trumble et al., 1998; Vickerman et al., 2002a,b). Selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}) are assimilated from soils by plants and by enzymatic reduction to organic selenocompounds, primarily selenomethionine (Birringer et al., 2002; Whanger, 2002). In insects and plants, organic Se is further converted to selenocysteine, which is co-translationally incorporated into selenoproteins (Ellis and Salt, 2003; Whanger, 2002; Zhou et al., 1999). Dietary selenate was assimilated by the larval

beet armyworm, *Spodoptera exigua* (Hübner), to selenite and selenomethionine (Vickerman et al., 2004).

Se levels in pest insects might play additional roles in the efficacy of biological control agents, such as the facilitation or inhibition of microbial infection. We examined this possibility by tracking the mortality of Se-supplemented cabbage loopers, *T. ni*, to the baculovirus AcMNPV. Fortuitously, a colony of the lepidopteran pest insect *T. ni* has been maintained at our laboratory for many generations on an artificial diet with no intentional effort to ensure adequate dietary Se. We determined that supplementation of the diet of these Se-depleted larvae with 25 ppm or more sodium selenite resulted in delayed growth of larvae as compared with those individuals fed less than 25 ppm which developed normally. We found that supplementation of the diet of these larvae with 5 or 10 ppm sodium selenite significantly boosted survival to subsequent baculovirus infection when larvae were fed Se in the later instars (Se/Se or NonSe/Se). This suggests that the presence of Se in larval tissues during viral infection lowers the susceptibility of larvae, particularly in initial mortality.

The mechanism whereby dietary Se-supplementation boosts resistance of *T. ni* larvae to baculovirus infection is unknown. Se depletion and repletion by dietary modification is known to alter the susceptibility to viral infection in vertebrates (Beck et al., 2004). In mammals, Se is a cofactor required for the activity of a number of selenoenzymes involved in the stress response (e.g., glutathione reductase, or thioredoxin reductase) which maintain nominal intracellular redox homeostasis, and which contribute to a more robust antimicrobial, specifically antiviral, defense (Beck et al., 2004). AcMNPV infection of a *T. ni* derived cell line resulted in oxidative stress and the depletion of cellular pro-oxidant levels, as measured by elevated lipid hydroperoxides, protein carbonyls, and reduced glutathione levels (Wang et al., 2001). Thioredoxin peroxidase expression is induced in tissues of larval silkworm, *Bombyx mori* L. 1758, by infection with the baculovirus BmNPV (Lee et al., 2005). Therefore, Se deficiency could result in increased oxidative stress in larval tissues, particularly the midgut (Hoover et al., 2000) or hemocytes (Trudeau et al., 2001; Washburn et al., 1996), and could impair the response against AcMNPV or other baculoviruses.

The only known function for the essential micronutrient Se in animals is as a redox cofactor co-translationally incorporated into selenoproteins as the amino acid residue selenocysteine (Beck et al., 2004). At least four selenoproteins have been identified in the genome of the dipteran *Drosophila melanogaster* Meigen (Castellano et al., 2001). However, none of the enzymes responsible for redox homeostasis identified in insects (i.e., glutathione reductase, thioredoxin reductase) are dependent upon Se for activity, as they are in mammals (Bauer et al., 2003; Kanzok et al., 2001; Lee et al., 2005; Missirlis et al., 2002; Singh et al., 2001). Deletion of the *Drosophila* translation elongation factor SelB, which eliminates selenoprotein synthesis entirely by blocking co-translational insertion of selenocysteine, did not affect longevity or survival following an oxidative insult (Hirosawa-Takamori et al., 2004). In contrast, disruption of the synthesis of the *Drosophila* selenoproteins dselK and dselH resulted in embryonic and larval lethality accompanied by deficits in intracellular antioxidants and in the accumulation of lipid peroxides (Morozova et al., 2003). Therefore, given that the role for Se in the insect oxidative stress response is subject to debate, we posit that one or more of the remaining selenoprotein activities may have a role in limiting baculovirus infection in *T. ni* and other lepidopteran larvae. Characterization of these selenoproteins will be the subject of a future report.

We have established that dietary supplementation with selenite significantly boosts larval *T. ni* resistance to the baculovirus AcMNPV in a dose-dependent manner. Soil Se levels exhibit considerable spatial variability (Dhillon and Dhillon, 2003). Assimilation and bioaccumulation of Se also differs among crops, weeds, herbivores, and predators (Jensen and Trumble, 2003). Our results suggest that soil Se levels, and the levels of other micronutrients, could influence the response of herbivorous insects to other biological control agents, and could disrupt IPM strategies dependent upon biological control agents. Selenate supplied systemically to plants has recently been suggested as a feeding deterrent, in effect a biological control, for the green peach aphid, *Myzus persicae* (Sulzer) (Hanson et al., 2004). Our results suggest that such supplementation might actually benefit herbivorous insects not deterred by high Se levels by increasing their resistance to microbial infection. Our results also suggest that provision of adequate dietary Se, and perhaps other micronutrients would improve the fitness and disease resistance of mass-reared beneficial insects used in augmentative release biological control programs. Additionally, in vivo bioassays evaluating insecticidal agents such as toxins or pathogens which were conducted using artificial diets containing suboptimal levels of Se or other micronutrients may need to be revisited. Finally, our results suggest that the type and level of specific soil micronutrients taken up by crops, and bioaccumulated to higher trophic levels may be adversely impacting the efficacy of some microbial biological control agents in a locality-specific manner.

Acknowledgments

We thank Steve Morris of the University of Missouri Research Reactor for determination of pupal Se content and Tom Coudron (USDA Agricultural Research Service) for reviewing an earlier draft. We thank John

Willenberg, Darrell Davis, Cindy Goodman, James Smith, Larry Brown, Brad Vickers, Delisa Butler, and Quezya Miner for technical assistance. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. All programs and services of the US Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

References

Anon, 2001. USDA Nutritional Database for Standard Reference Release 14.

Banuelos, G.S.V.D.B., Trumble, J.T., Shannon, M.C., Davis, C.D., Finley, J.W., Mayland, H.F., 2002. Biotransfer possibilities of selenium from plants used in phytoremediation. Int. J. Phytoremediation 4, 315–331.

Bauer, H., Gromer, S., Urbani, A., Schnolzer, M., Schirmer, R.H., Muller, H.M., 2003. Thioredoxin reductase from the malaria mosquito *Anopheles gambiae*: comparisons with the orthologous enzymes of *Plasmodium falciparum* and the human host. Eur. J. Biochem. 270, 4272–4281.

Beck, M.A., Handy, J., Levander, O.A., 2004. Host nutritional status: the neglected virulence factor. Trends Microbiol. 12, 417–423.

Birringer, M., Pilawa, S., Flohe, L., 2002. Trends in selenium biochemistry. Nat. Prod. Rep. 19, 693–718.

Castellano, S., Morozova, N., Morey, M., Berry, M.J., Serras, F., Corominas, M., Guigo, R., 2001. In silico identification of novel selenoproteins in the *Drosophila melanogaster* genome. EMBO Rep. 2, 697–702.

Clarke, T.E., Clem, R.J., 2003. Insect defenses against virus infection: the role of apoptosis. Int. Rev. Immunol. 22, 401–424.

Dhillon, K.S., Dhillon, S.K., 2003. Distribution and management of seleniferous soils. Adv. Agron. 79, 119–184.

Dietrich, C.P., Nadar, H.B., Toma, L., de Azambuja, P., Garcia, E.S., 1987. A relationship between the inhibition of heparin sulfate and chondroitin sulfate synthesis and the inhibition of molting by selenite in the hemipteran *Rhodnius prolixus*. Biochem. Biophys. Res. Commun. 146, 652–658.

Ellis, D.R., Salt, D.E., 2003. Plants, selenium and human health. Curr. Opin. Plant Biol. 6, 273–279.

Hanson, B., Garifullina, G.F., Lindblom, S.D., Wangeline, A., Ackley, A., Kramer, K., Norton, A.P., Lawrence, C.B., Pilon-Smits, E.A.H., 2003. Selenium accumulation protects *Brassica juncea* from invertebrate herbivory and fungal infection. New Phytol. 159, 461–469.

Hanson, B., Lindblom, S.D., Loeffler, M.L., Pilon-Smits, E.A.H., 2004. Selenium protects plants from phloem-feeding aphids due to both deterrence and toxicity. New Phytol. 162, 655–662.

Hirosawa-Takamori, M., Chung, H.R., Jackle, H., 2004. Conserved selenoprotein synthesis is not critical for oxidative stress defense and the lifespan of *Drosophila*. EMBO Rep. 5, 317–322.

Hoover, K., Washburn, J.O., Volkman, L.E., 2000. Midgut-based resistance of *Heliothis virescens* to baculovirus infection mediated by phytochemicals in cotton. J. Insect Physiol. 46, 999–1007.

Hughes, P.R., van Beek, N.A.M., Wood, H.A., 1986. A modified droplet feeding method for rapid assay of *Bacillus thuringiensis* and baculoviruses in noctuid larvae. J. Invertebr. Pathol. 48, 187–192.

Jensen, P., Trumble, J.T., 2003. Ecological consequences of bioavailability of metals and metalloids in insects. Recent Res. Dev. Entomol. 42, 1–17.

Kanzok, S.M., Fechner, A., Bauer, H., Ulschmid, J.K., Muller, H.M., Botella-Munoz, J., Schneuwly, S., Schirmer, R.H., Becker, K., 2001. Substitution of the thioredoxin system for glutathione reductase in *Drosophila melanogaster*. Science 291, 643–646.

Lee, K.S., Kim, S.R., Park, N.S., Kim, I., Kang, P.D., Sohn, B.H., Choi, K.H., Kang, S.W., Je, Y.H., Lee, S.M., Sohn, H.D., Jin, B.R., 2005. Characterization of a silkworm thioredoxin peroxidase that is induced by external temperature stimulus and viral infection. Insect Biochem. Mol. Biol. 35, 73–84.

McIntosh, A.H., Ignoffo, C.M., 1983. Restriction endonuclease patterns of three baculoviruses isolated from species of *Heliothis*. J. Invertebr. Pathol. 41, 27–32.

McKown, D.M., Morris, J.S., 1978. A rapid method for measuring selenium in biological materials. J. Radioanal. Chem. 43, 409.

Missirlis, F., Ulschmid, J.K., Hirosawa-Takamori, M., Gronke, S., Schafer, U., Becker, K., Phillips, J.P., Jackle, H., 2002. Mitochondrial and cytoplasmic thioredoxin reductase variants encoded by a single *Drosophila* gene are both essential for viability. J. Biol. Chem. 277, 11521–11526.

Morozova, N., Forry, E.P., Shahid, E., Zavacki, A.M., Harney, J.W., Kraytsberg, Y., Berry, M.J., 2003. Antioxidant function of a novel selenoprotein in *Drosophila melanogaster*. Genes Cells 8, 963–971.

O'Reilly, D.R., Brown, M.R., Miller, L.K., 1992. Alteration of ecdysteroid metabolism due to baculovirus infection of the fall armyworm *Spodoptera frugiperda*: host ecdysteriods are conjugated with galactose. Insect Biochem. Mol. Biol. 22, 313–320.

Popham, H.J.R., Shelby, K.S., Brandt, S.L., Coudron, T.A., 2004. Potent virucidal activity against HzSNPV in larval *Heliothis virescens* plasma. J. Gen. Virol. 85, 2225–2261.

Rolff, J., Siva-Jothy, M.T., 2003. Invertebrate ecological immunology. Science 301, 472–475.

SAS Institute Inc., 1999. SAS/STAT User's Guide, Version 8. SAS Institute Inc., Cary, NC.

Schultz, J.C., 2002. Biochemical ecology: how plants fight dirty. Nature 416, 267.

Singh, S.P., Coronella, J.A., Benes, H., Cochrane, B.J., Zimniak, P., 2001. Catalytic function of *Drosophila melanogaster* glutathione *S*-transferase DmGSTS1-1 (GST-2) in conjugation of lipid peroxidation end products. Eur. J. Biochem. 268, 2912–2923.

Slavicek, J.M., Popham, H.J.R., Riegel, C.I., 1999. Deletion of the *Lymantria dispar* multicapsid nucleopolyhedrovirus ecdysteroid UDP-glucosyl transferase gene enhances viral killing speed in the last instar of the Gypsy Moth. Biol. Control 16, 91–103.

Trudeau, D., Washburn, J.O., Volkman, L.E., 2001. Central role of hemocytes in *Autographa californica* M nucleopolyhedrovirus pathogenesis in *Heliothis virescens* and *Helicoverpa zea*. J. Virol. 75, 996–1003.

Trumble, J.T., Kund, G.S., White, K.K., 1998. Influence of form and quantity of selenium on the development and survival of an insect herbivore. Environ. Pollut. 101, 175–182.

Vickerman, D.B., Shannon, M.C., Banuelos, G.S., Grieve, C.M., Trumble, J.T., 2002a. Evaluation of *Atriplex* lines for selenium accumulation, salt tolerance and suitability for a key agricultural insect pest. Environ. Pollut. 120, 463–473.

Vickerman, D.B., Trumble, J.T., 1999. Feeding preferences of *Spodoptera exigua* in response to form and concentration of selenium. Arch. Insect Biochem. Physiol. 42, 64–73.

Vickerman, D.B., Trumble, J.T., George, G.N., Pickering, I.J., Nichol, H., 2004. Selenium biotransformations in an insect ecosystem: effects of insects on phytoremediation. Environ. Sci. Tech. 38, 3581–3586.

Vickerman, D.B., Young, J.K., Trumble, J.T., 2002b. Effect of selenium-treated alfalfa on development, survival, feeding and oviposition preferences of *Spodoptera exigua* (Lepidoptera: Noctuidae). Environ. Entomol. 31, 953–959.

Wang, Y., Oberley, L.W., Murhammer, D.W., 2001. Evidence of oxidative stress following the viral infection of two lepidopteran insect cell lines. Free Radic. Biol. Med. 31, 1448–1455.

Washburn, J.O., Kirkpatrick, B.A., Volkman, L.E., 1996. Insect protection against viruses. Nature 383, 767.

Whanger, P.D., 2002. Selenocompounds in plants and animals and their biological significance. J. Am. Coll. Nutr. 21, 223–232.

Wilkinson, J.D., Morrison, R.K., Peters, P.K., 1972. Effect of Calco Oil Red N-1700 dye incorporated into a semiartificial diet of the imported cabbageworm, corn earworm, and cabbage looper. J. Econ. Entomol. 36, 264–268.

Zhou, X., Park, S.I., Moustafa, M.E., Carlson, B.A., Crain, P.F., Diamond, A.M., Hatfield, D.L., Lee, B.J., 1999. Selenium metabolism in *Drosophila*. Characterization of the selenocysteine tRNA population. J. Biol. Chem. 274, 18729–18734.